

tendency there may have been in textbooks to present BAYER's hypothesis as representing facts.

They discuss the theories and suggestions of four men: (1) the well known theory of BAYER involving formaldehyde as an intermediate product; (2) the suggestion of VAN'T HOFF that assimilation consists of two parts, a photochemical reaction and an enzyme reaction; (3) SIEGFRIED's suggestion that carbon dioxide may form carbamino groups with the protoplasm of the plant cell and that the photochemical reaction may then occur in a complex carbon compound; and (4) WILLSTÄTTER's theories which, so far as they are new, are regarded by the reviewers as rather wild, the most reasonable one suggested by him being merely a repetition of SIEGFRIED's suggestion.

In the end it appears that we have at present no satisfactory theory of the changes that take place between the entrance of CO_2 into the plant and the production of carbohydrates.

Although deploring the lack of coordination among the various workers, and the tendency of botanists to accept without question the suggestions of physicists and chemists as to the nature of plant processes, the reviewers conclude that plant physiology is developing into an exact science, utilizing the experiences of the fundamental sciences, physics and chemistry, but having working principles and methods of its own. That it will thus be of great service in plant production requires no prophetic vision.—GEORGE B. RIGG.

Studies on oxidases.—In connection with his work on plant oxidases, BUNZELL⁷ has published results of an investigation of the effect of hydrogen-ion concentration, C_h , on oxidase activity. Using his own simplified oxidase apparatus to measure oxidation and the gas chain to measure hydrogen-ion concentration, he finds that the oxidase activity of several kinds of material from potato tubers is completely inhibited by a C_h of $2.0-2.8 \times 10^{-4}$. The various concentrations were obtained by adding sodium hydroxide and acetic acid in various proportions, or either one alone, to mixtures of the plant material and pyrocatechin.

It is worth noting here that the two together constitute a true buffer solution capable of maintaining a fairly constant hydrogen-ion concentration, but that neither one alone suffices. Consequently, if there is a tendency for the acidity to increase in the Bunzell apparatus, as suggested by ROSE⁸ in 1915, conditions are not comparable in the different mixtures. Those containing the true buffer solution will have practically a constant C_h throughout the course of the experiment, while those containing only sodium hydroxide or acetic acid will have a C_h which is larger at the end than at the beginning. The

⁷ BUNZELL, H. H., The relationship existing between the oxidase activity of plant juices and their hydrogen-ion concentration, with a note on the cause of oxidase activity in plant tissue. *Jour. Biol. Chem.* 28:315-333. 1916.

⁸ ROSE, D. H., Oxidation in healthy and diseased apple bark. *BOT. GAZ.* 60: 55-65. 1915.

latter condition holds true also for the controls, containing only water, pyrocatechin, and plant material. Furthermore, if the C_h changes during the experiment and only the initial concentration is determined, as in BUNZELL'S work, no very accurate conclusion can be drawn as to the effect of this factor on oxidase activity.

BUNZELL finds the inhibiting concentration for tulip tree material to lie between 1.58 and 5.02×10^{-3} , and for the magnolia between 3.5×10^{-3} and 8.91×10^{-4} . He considers that his results show "that the acid sensitiveness figure is a rather fixed number for any particular genus." He says also that it even seems "that the acid sensitiveness constant is the same or nearly the same for different genera (tulip and magnolia) of the same family (Magnoliaceae)." An analysis of his table III shows in general that the less the natural acidity of the plant material the lower the C_h necessary to cause total inhibition of its oxidase activity. This relation does not seem to hold in all cases, possibly because the various degrees of acidity used were too far apart to establish the inhibiting concentration with any great degree of accuracy.

If further work should prove such a relation general, new force will be added to the suggestions of BUNZELL and others that there is a distinct oxidase for each plant or group of closely related plants; not necessarily because they are protein in nature, however, as BUNZELL supposes. They may resemble each other in plants of the same family; they may show various properties of proteins, such as denaturing by acids, alcohol, and heat, and still be something quite different from proteins. BAYLISS suggests, on the basis of work by BACH and CHODAT and others, that oxidases are merely some form of iron copper or manganese kept in a disperse condition by various colloids. If these colloids are proteins the action of acids, for example, removes them as dispersing agents and allows the oxidases to precipitate. As a result of absorption, the two may come down together as a single precipitate which gives both protein and oxidase reactions without ever having existed as a real compound in the living plant. Such a hypothesis, however, fails to apply to peroxidases, for these, according to BEHRING, ASO, and BACH and CHODAT, are very little affected by heat. BACH and CHODAT also found that horseradish peroxidase when carefully purified contains no iron or manganese.

In connection with BUNZELL'S "acid sensitiveness figure," the question arises whether the inhibition he noted was all due to acidity. When a buffer solution of any sort is used to establish a definite hydrogen-ion concentration, elements are added which in the quantity used may be entirely foreign to the plant and productive of anomalous results. Illustrations of this are seen in BUNZELL'S table III. For example, extract of potato peeling with a natural C_h of 1.02×10^{-6} (no buffer solution being present) caused 22 per cent more oxidation than the same extract when a buffer solution was present and the C_h practically the same (1.04×10^{-6}). Even more marked are the results with potato sprouts, for with the C_h just about the same whether the buffer solution were present or not, they gave 16 per cent more oxidation without

it than with it. The data presented for "tulip tree leaves 1915" and "scaled tulip tree buds" show that when the solution in the oxidase apparatus had the natural reaction of the plant material, the oxidations were respectively 6.6 and 12 per cent greater than when the C_h , established by a buffer solution, was actually less than the natural C_h . In such a case it seems evident that some factor other than the hydrogen-ion concentration was effective as an inhibitor. The possibility that other ions play a part is indicated by work now being carried on by KRAYBILL and the writer.

The paper concludes with a brief review of the evidence, obtained by BUNZELL and others, of an increased oxidase activity in the leaf tissue in the case of physiological disturbances, and the possible meaning of such an increase. No mention, however, is made of work by ROSE on healthy and diseased apple bark in which it was shown that there is a much greater oxidase activity in the latter, correlated with a lower hydrogen-ion concentration.

REED,⁹ in a paper published about the same time as BUNZELL's, puts the inhibiting C_h for oxidase of potato extract at 5.5×10^{-4} (slightly higher than the $2.0-2.8 \times 10^{-4}$ found by BUNZELL), and for that of Red Astrachan apples at $5.0-7.0 \times 10^{-4}$. His statement that these concentrations are much lower than those given by previous investigators fails, however, to take account of BERTRAND's report¹⁰ in 1907, that a $n/5000$ solution of sulphuric acid ($C_h = 5 \times 10^{-4}$) completely inhibited oxidation by sap of the lac tree. REED's results would have meant more if he had measured oxidation by the BUNZELL apparatus rather than by the relatively inaccurate method of noting color changes, even though the BUNZELL apparatus, because of the poorly understood effects of hydrogen-ion and other inhibitors, leaves much to be desired in the way of accuracy.

One point is well made in this paper, namely, that plant extracts have an acid absorbing power which must cause inaccuracy in interpreting results obtained by adding buffer solutions to them if such results are not checked by careful determinations of the hydrogen-ion concentration. He found that when a given volume of 0.01 molar HCl was added to an equal volume of potato extract, the hydrogen-ion concentration, which should have been 5×10^{-3} if the potato extract acted like water, was actually only 5×10^{-4} . This decrease in acidity he thinks is due to proteins present in the extract as well as other amphoteric electrolytes, including probably phosphates and carbonates.

It is unfortunate, to say the least, that the authors of these papers have failed to cite adequately the literature pertinent to the phase of the subject with which they are dealing. Each has made a definite contribution to our

⁹ REED, G. B., The relation of oxidase reactions to changes in hydrogen-ion concentration. *Jour. Biol. Chem.* 27:299-303. 1916.

¹⁰ BERTRAND, G., *Bull. Soc. Chim. France* 1:1120. 1907.

knowledge of the factors affecting oxidase activity, but the true value of this contribution would have been better shown by a fuller reference to other work.

KASTLE and BUCKNER¹¹ report experimental proof that phenolphthalein can be oxidized in the living plant. This they take to mean that free active oxygen is present in the tissues, apparently overlooking the possibility that combined oxygen might have caused the results observed. The reagent used, on oxidation, yields phenolphthalein, which is easily recognized by the pink color it gives with alkalies. When this test was applied to stalks of Indian corn which had been injected with the reagent, the pink color was found localized in the fibrovascular bundles of the stem and leaves. It was not found in the tassel, although lower down, close to the point of injection, there had been some diffusion into the cells adjoining the fibrovascular bundles. Similar results as to place of oxidation were obtained with okra.

The method here used offers a means of attacking the problem of oxidation in plants which should yield other valuable results if further developed and applied to a wider series of plants. It would be worth while to try whether phenolphthalein can be oxidized in the living plant when used in neutral or acid solution, and if so whether the oxidation is localized in particular cells or tissues. Such a test would allow for the effect of reaction (acidity or alkalinity), a factor known to be of great importance, not only in oxidation processes, but also in other processes carried on in living tissues. The effect of reaction might also be studied in acid fruits and in tissues affected by "physiological diseases" or by diseases due to bacterial or fungus parasites. In several cases such tissues have been found to be less acid than healthy ones, but little is known concerning variations in reaction within the tissues themselves.—D. H. ROSE.

Experiments in girdling.—A contribution by HIBINO¹² is of interest both to plant physiologists and horticulturists, since it will aid in furnishing a more definite chemical basis for the interpretation of the behavior of girdled plants. In the past there has been no lack of references to the accumulation of elaborated foods above the girdles; it is certainly worth while to have some definite determinations of these compounds and their relative quantities.

Five types of girdling were tried on *Cornus contraversa* Hemsl. These consisted in (1) removing a complete ring of bark, (2) removing a complete ring of bark and some of the wood, (3) removing half a ring of bark, (4) removing half a ring of bark and wood, and (5) boring completely through the wood. The wounds were left unprotected. The last three methods of treating the material resulted in responses similar to the untreated controls in nearly all cases.

The general external results noted are those commonly recorded in girdling experiments. The main interest of the present paper centers in the presenta-

¹¹ KASTLE, J. H., and BUCKNER, G. DAVIS, Evidence of the action of oxidases within the living plant. Jour. Amer. Chem. Soc. 39:479-482. 1916.

¹² HIBINO, SHIN-ICHI, Effekt der Ringelung auf die Stoffwanderung bei *Cornus controversa* Hemsl. Jour. Coll. Sci. Imp. Univ. Tokyo 39:1-40. pls. 1, 2. 1917.